REMARKS

Claims 1-5, 7-13, 27, 28, 32-34, 51 and 52 are currently pending in the application. Claims 77 and 78 are withdrawn. Claims 1-4, 7-9, 11, 27, 28, 30, 32-34 and 51 are amended. Claims 79 and 80 have been newly added. The amendments find support in the specification and are discussed in the relevant sections below. No new matter is added.

Elections/Restrictions

The Office Action states that newly submitted claims 77 and 78 are directed to an invention that is independent or distinct from the invention originally claimed. The Office Action states that "claims 77 and 78 the addition or removal of the moiety promotes dissociation and further requires detecting dissociation and the originally filed claims do no require these limitations (see also reasons stated in the restriction requirement filed June 18, 2002)."

Applicants withdraw claims 77 and 78. However, Applicants respectfully disagree with the assertion of the Office Action that these claims are directed to an invention that is independent and distinct from the invention originally claimed. Applicants submit that detecting dissociation requires the same steps as detecting association. Applicants submit that this is largely a semantic issue: for example, measuring light will yield essentially the same answer as measuring darkness. Likewise, methods which allow the detection of dissociation of polypeptides can be used to detect association of polypeptides. Even if they are treated as separate means of detection, Applicants submit that an search for art related to association of polypeptides will yield art on dissociation, and therefore assert that it would not pose a serious burden on the examiner.

Applicants submit that newly presented claims 79 and 80 have been introduced which recite "wherein addition or removal of said moiety **promotes association** of said one or more binding partner polypeptides from the corresponding one or more tagged binding partner polypeptides".

Claim Objections

Claims 2-4, 7-9, 11, 27, 28, 30, 32-34, 51 and 52 are objected to because of they depended from non-elected claims, specifically claims 77 and 78.

With this amendment, all claims with improper dependence on non-elected claims have been amended to depend only from elected claims. Specifically, Applicants have amended claims 2-4, 7-9, 11, 27, 28, 30, 32-34 and 52 so that they no longer depend from non-elected claims 77 and 78. Claim 51, however, is an independent claim and does not depend from any non-elected claims. As such, claim 51 has not been amended. Applicants respectfully request withdrawal of the claim objections and reconsideration of the claims. As such, new claims 79 and 80 are within the scope of the elected claims.

Claim Rejections

Rejection under 35 U.S.C. §112

Claims 1-5, 7-13, 27, 28, 30, 32-34, 51 and 52 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Office Action states that claims 1 and 51 are rejected as being incomplete for omitting essential steps such as the immobilization of the binding partner polypeptides and a wash step. With regard to washing and immobilization steps, the Office Action states:

> "without the above steps the instantly recited claims do not work because the detector molecule binds to the tag binding partner and regardless if enzyme activity occurs the detection molecule will bind to the tag binding partner and thus a positive signal will be detected. Further a wash step would have to be performed after the addition of the detection molecule to determine if binding of the detection molecule has occurred and also to remove excess detection molecule."

The Office Action further states that it is unclear how to "detect binding between tagged binding partner polypeptides and the binding partner polypeptides by the addition of the detector molecule. The detector molecule as recited binds to the tagged binding partner and regardless if the tagged binding partner binds the binding partner polypeptide or not the detector molecule

will bind to the tagged binding partner and thus a positive signal will always be detected. Claims 1 and 51 as instantly recited will not work."

Applicants respectfully disagree. Applicants note that neither an immobilization or washing step is necessary for the claimed method to work. The Specification describes, for example, embodiments in which FCS or FRET are used to detect association. The use of these techniques can effectively avoid the need for immobilization or washing steps. Applicants submit that the Specification describes a number of non-limiting examples which do not require an immobilization step or a washing step:

> "The one or more binding partner polypeptides can also be tagged. Further, one or more binding partner polypeptides and said one or more tagged binding partner polypeptides can be tagged with one or more fluorescent molecules. **Detecting can then comprise monitoring the presence or absence of fluorescent resonance energy transfer (FRET).**" (emphasis added, p. 9 line 19 – p. 10 line 2)
>
> "**The interaction between the binding partners can then be followed in solution using fluorescence resonance energy transfer (FRET) or fluorescence polarization (FP) based assays**, where one or both of the partners involved in the interaction are labeled with a fluorophore. Alternatively, immobilized formats can be used, where one binding partner polypeptide is fixed to a solid surface and the binding of a second, fluorescently labeled partner is detected." (emphasis added, p. 21, line 17 – p.22 line 3)
>
> "**In solution phase assays**, the number of enzymes that can be measured at one time depends on the number of fluorophores able to be simultaneously detected by a suitable instrument." (emphasis added, p. 22, lines7-9)
>
> "**In the solution phase assays**, at least two different colored labels are needed for FRET, and multiple colors are necessary to perform multiple FP assays in the same tube. Furthermore, in order to monitor the association of modification dependent binding proteins using FRET or FP, the fluorescent labels must be able to be specifically placed in the correct location and orientation." (emphasis added, p.77, line 24 – p.78, line 4):
>
> "**Solution Phase FRET Assay for Src tyrosine kinase**
> In a similar way to the ZAP labeling procedure, the TCR zeta chain domain fused to the 4HA 20 coiled-coil peptide can be labeled with a 5HB peptide carrying rhodamine. The two binding domains, ZAP7O and TCR, labeled with fluorescein and rhodamine respectively, can be used to monitor the activities of kinases or

phosphatases acting to phosphorylate or dephosphorylate the TCR zeta chain. An SH2 domain labeled with fluorescein can interact with phosphorylated TCRζ chain labeled with rhodamine. The fluorophores will then be close enough for FRET to occur between fluorescein and rhodamine (see Figure 4). **Rhodamine will accept energy from fluorescein when in close proximity resulting in a loss of emission from the fluorescein that can be followed using a fluorimeter or fluorescent microtitre plate reader such as the BMG Polarstar Galaxy.**"

"SH2 and TCR domains can be mixed in a suitable buffer (e.g. 20mM Tris-HC1 pH7.2, 1mM ATP, 10mM MgCl2, 0.04mM EDTA, 0.003% (v/v) Brij 35, 0.04mg/ml BSA, 0.08% (v/v) β-mercaptoethanol). Src enzyme can be added to start the reaction. Phosphorylation of the TCR zeta chain is followed by the change in fluorescein quench due to FRET."

"Alternatively, ZAP7O-labeled with fluorescein via the FJ coil can be used with a chemically labeled synthetic TCR peptide coupled to rhodamine, and **enzyme activity monitored again by acceptor gain or donor loss in FRET**."

Solution Phase FRET Assay for Tyrosine Phosphatases

Measurement of phosphatase activity can be shown using a phosphatase, YOP from Yersinia pestis, (Upstate Biotechnologies). Phosphatase activity can be detected with the ZAP7O labeled with FJ peptide-fluorescein and a rhodamine labeled peptidic mimic of the phosphorylated TCRζ chain:
(RCKFSRSAEPPAYQQGQNQLY(p)NELNLGRREEY(p)DVLD) (SEQ ID NO:40) labeled with rhodamine at the thiol position.

"Reactants can be mixed in a suitable buffer (e.g. 50mM Tris-HC1 pH7.4 including 150mM NaC1 and 0.2%(v/v) Tween 20) and allowed to equilibrate. **Interaction will occur only between the phosphorylated peptide and the SH2 domain, bringing the fluorophores close enough for FRET to occur**. The unphosphorylated peptide will not bind to the SH2 domain. **Addition of the phosphatase will disrupt the interaction by removing the phosphate moieties, thereby reducing FRET.**" (emphasis added, p.96, line 18 – p.98, line 12)

Additional disclosure of embodiments which do not require immobilization relates to, for example, fluorescence correlation spectroscopy (FCS) (p. 75, line 3 – p. 76, line 2), which relies on the measurement of the rate of diffusion of a label. It is noted that FCS can be performed with a sincle labeled species. Further, as will be apparent to one skilled in the art, the diffusion rate of an immobilized sample would be zero, such that the use of an immobilized sample would not yield meaningful measurements of polypeptide association. Therefore, by definition, use of FCS

as a means of measuring enzyme activity contemplates the use of polypeptides which are not immobilized.

Therefore, Applicants submit that immobilization and washing are not required steps but, rather, are optional steps, and further submit that there is ample support within the Specification for embodiments which do work, without requiring washing steps and/or immobilization steps. Therefore, Applicants submit that the steps of washing and immobilization are optional steps and, as such, claims 1 and 51 are definite in this regard. As such, Applicants respectfully request withdrawal of the §112, second paragraph rejection and reconsideration of claims 1 and 5.

With respect to Claim 1, the Office Action further states that "claim 1, part (ii) the recitation "correspond to said tagged binding partner polypeptides" is vague and indefinite." The Office Action asks "how does the binding partner polypeptide correspond to the tagged binding partner polypeptide? Does it comprise similar sequences, does it bind to the tagged binding partner polypeptide or is there another binding partner polypeptide? See also deficiency found in claim 51."

With this Amendment, Applicants have amended step (ii) of claim 1 and claim 51 to claim:

"(ii) one or more binding partner polypeptides that **bind to** said one or more tagged binding partner polypeptides of (i);"

Applicants submit that claim 1, as amended, no longer recites "correspond to said tagged binding partner polypeptides." Applicants further submit that terms "associates" and "binds" are defined in the instant application:

> As used herein, the term "**associates**" or "**binds**" **refers to a binding partner polypeptide as described herein and its binding partner having a binding constant sufficiently strong to allow detection of binding by detection methods, which are in physical contact with each other and have a dissociation constant (Kd) of about 10μM or lower**. The contact region may include all or parts of the two molecules. Therefore, the terms "substantially dissociated" and "dissociated" or "substantially unbound" or "unbound" refer to the absence or loss of contact between such regions, such that the binding constant is reduced by an amount which produces a discernable change in a signal compared to the bound state, including a total absence or loss of contact, such that the proteins are completely separated, as well as a partial absence or loss of

> contact, so that the body of the proteins are no longer in close proximity to each other but may still be tethered together or otherwise loosely attached, and thus have a dissociation constant greater than 10μM (Kd). In many cases, the Kd will be in the mM range." (emphasis added, p. 18, line 15 – p. 19, line 5).

Therefore, Applicants submit that the instant claim is definite in this regard.

The Office Action states that "claim 1 is vague and indefinite because it is unclear if the moiety is in the mixture floating around and the enzyme causes it to attach to the polypeptide or is the moiety part of the polypeptide to begin with and the enzyme causes a conformational change or release of the moiety."

Applicants respectfully disagree. Applicants submit that the moiety's disposition is dependent on the type of enzyme used, and is clear to the skilled artisan. Applicants further submit that the specification fully supports both addition and removal of moieties.

Many enzymes can be used with the methods of the present invention. Non-limiting examples are discussed in the Specification, e.g., p. 43, lines 1-11, which states: "The invention requires the presence of a modifying enzyme which catalyzes either the addition or removal of a modifying group (moiety) or proteolysis. Preferably, the enzyme is one of the following enzymes: a protein phosphatase, a protein kinase, a carbohydrate transferase (e.g., a UDP-N-Acetylglucosamine-Dolichyl-phosphate-N-acetylsglucosamine phosphotransferase or an O-GlcNAc transferase), a ubiquitin activating enzyme El, a ubiquitin conjugating enzyme E2, a ubiquitin conjugating enzyme Ubc9, a ubiquitin protein ligase E3, a poly (ADP-ribose) polymerase, a fatty acyl transferase (e.g., a peptide N-myristoyltransferase), an NAD:Arginine ADP ribosyltransferase, and a protease. A range of kinases, phosphatases, and other modifying enzymes are available commercially (e.g. from Sigma, St. Louis, MO; Promega, Madison, WI; Boehringer Mannheim Biochemicals, Indianapolis, IN; New England Biolabs, Beverly, MA; and others). Alternatively, such enzymes may be prepared in the laboratory by methods well known in the art."

Applicants submit that the status of the moiety depends upon the action of the enzyme, but that there is nothing indefinite about this, as one skilled in the art will know whether the moiety is free in solution or attached to a polypeptide to begin with. This is recognized, e.g., in

the Examples sections of the Specification. For example, within the section titled Solution Phase FRET Assay for Src tyrosine kinase, , the reaction takes place in "a suitable buffer (e.g. 20mM Tris-HCl pH7.2, **1mM ATP**, 10mM MgCl2, 0.04mM EDTA, 0.003% (v/v) Brij 35, 0.O4mg/ml BSA, 0.08% (v/v) β-mercaptoethanol." (emphasis added, p. 103, lines 3-5). In contrast, within the section titled Solution Phase FRET Assay for Tyrosine Phosphatases it is described: "**Phosphatase activity can be detected with** the ZAP7O labeled with FJ peptide-fluorescein and a rhodamine labeled peptidic mimic of the **phosphorylated TCRζ chain**." (emphasis added, p. 103, lines 9-10) "Addition of the **phosphatase will disrupt the interaction by removing the phosphate moieties**, thereby reducing FRET." (emphasis added, p. 103, lines 16-17). Furthermore, the section titled Immobilized Tyrosine Phosphatase Assay uses a "**phosphorylated peptide** mimicking the critical structure of the TCRζ chain." (emphasis added, p. 104, lines 9-10). It will be clear to the skilled artisan that the disposition of the moiety depends upon the type of enzyme whose activity is to be monitored. As such, Applicants submit that that claims, as written, are definite and clear in this regard.

The Office Action states that "claim 5 is vague and indefinite because it is unclear how the rate of diffusion of the fluorescent molecule is monitored." The Office Action further states: "it is unclear what media the fluorescent molecule is in. Is it in a solution or a gel? Further, the instantly recited claims could be performed in a test tube and if so it is unclear if a diffusion rate could be performed in this environment. Please clarify."

Applicants submit that the method of diffusion measurement need not be recited in order for the claim to be definite. One skilled in the art will know how to measure diffusion or will be able to employ a method as described in the Specification. Applicants submit that measurement of diffusion is described within the instant application, for example, as follows:

> "Further embodiments of the present invention are not dependent on FRET. **For example the invention can make use of fluorescence correlation spectroscopy (FCS), which relies on the measurement of the rate of diffusion of a label** (see Elson and Magde, (1974) Biopolymers, 13:1; Rigler et al., (1992) in Fluorescence Spectroscopy: New Methods and Applications, Springer Verlag, pp.13-24; Eigen and Rigler, (1994) Proc. Natl. Acad. Sci. U.S.A., 91:5740; Kinjo and Rigler, (1995), Nucleic Acids Res., 23:1795)."

> "In FCS, **a focused laser beam illuminates a very small volume of solution**, of the order of 10^{-15} liter, which at any given point in time contains only one molecule of the many under analysis. The **diffusion of single molecules through the illuminated volume, over time, results in bursts of fluorescent light as the labels of the molecules are excited by the laser. Each individual burst, resulting from a single molecule, can be registered.**"
>
> "A labeled polypeptide will diffuse at a slower rate if it is large than if it is small. **Thus, multimerized polypeptides will display slow diffusion rates, resulting in a lower number of fluorescent bursts in any given time frame, while labeled polypeptides which are not multimerized or which have dissociated from a multimer will diffuse more rapidly**. Binding of polypeptides according to the invention can be calculated directly from the diffusion rates through the illuminated volume."
>
> **"Where FCS is employed, rather than FRET, it is not necessary to label more than one binding partner polypeptide**. Preferably, a single polypeptide member of the multimer is labeled. The labeled polypeptide dissociates from the multimer as a result of modification, thus altering the FCS reading for the fluorescent label." (emphasis added, p. 75, line 3 – p. 76, line 2)

Applicants submit that diffusion occurs and can be measured in any fluid sample, including a gel, so the medium is not critical to the definiteness of the claims. Similarly, the vessel in which the assay is performed need only be compatible with the selected method of measuring diffusion and need not be recited to render the claims definite. As the assay can be used to measure relative diffusion rates, Applicants submit that the medium in which the assay is measured is not directly relevant, so long as the medium permits the measurement of diffusion. Therefore, Applicants submit that claim 5, as written, is definite and as such request the withdrawal of the §112, second paragraph rejection of this claim.

The Office Action sates that "claim 12 is vague and indefinite because as recited the binding partner would comprise a fluorescent molecule, the tagged binding partner would comprise a fluorescent molecule and the detector molecule would comprise a reporter molecule. There would be labels and it is unclear how detection would be carried out with three labels."

Applicants respectfully disagree. Claim 12 depends from claim 11 which, in turn, depends from claim 1. Applicants submit that claim 12, as written, does not necessarily entail the use of three labels. As is described within the specification, a tag can be non-fluorescent:

"**The tag can be selected from the group consisting of a coiled-coil, an antigen, an antibody, a single chain antibody, a radioactive amino acid, a fluorescent molecule, a reporter enzyme, and biotin**" (emphasis added, page 10, lines 12-14).

"**A tag can comprise, but is not limited to an antigen or an epitope tag such as myc** (Roth et al., (1991) J. Cell Biol. 115:587); **HA, derived from influenza hemagglutinin protein** (Wilson et al., (1984) Cell 37:767; FLAG (U.S. Patent 4,793,004; 4,851,341); **IRS** (RYTRS or IRS antibodies available from Berkeley Antibody Company, Richmond CA, (BAbCO)); **AU1** (DTYRYI, antibodies available from BAbCO); **AU5** (TDFLYK, antibodies available from BAbCO); **glu-glu** (EEEEYMPME, antibodies available from BAbCO); KT3 (KPPTPPPEPET, antibodies available from BAbCO); **T7 Tag**^{TM} (antibodies available from Novagen, Madison, WI); **HSV TagTM** (antibodies available from Novagen); and S-TagTM (antibodies available from Novagen), **VSV-G** (antibodies available from Research Diagnostics, Inc., Flanders NJ), **and His Tag** (antibodies available from Research Diagnostics, Inc., Flanders NJ). **A tag can further comprise an antibody, a single-chain antibody, a coiled-coil region, sequence specific nucleic acid binding domains, such as DNA or RNA binding domains, SH3 or SH2 peptide interactions, metal chelating amino acids** (Porath et al., (1975) Nature 258:598; Lorinerdal et al., (1982) J. Appl. Biochem. 4:203; Sulkowski, (1985) Trends Biotechnol. 3:1; U.S. Patent No. 5,047,513), a fluorochrome, a fluorescent protein, including green-fluorescent proteins, blue-fluorescent proteins, and red-fluorescent proteins, **a molecule that quenches a fluorescent molecule, a radioactive amino acid or amino acids, a radioisotope, a radionuclide containing molecule, a reporter enzyme, such as luciferase** (Brasier et al., (1989) BioTechniques 7:1116), **chloramphenicol acetyltransferase (CAT)** (Gorman et al., (1982) Mol. Cell Biol. 2:1044), **horseradish peroxidase, alkaline phosphatase, glucose oxidase, hexokinase with glucose-6-phosphate dehydrogenase, or β-galactosidase** (An et al., (1982) Mol. Cell Biol. 2:1628), **or a biotin molecule and analogs and derivatives thereof.** Antigens such as, but not limited to, glutathione S-transferase (GST), green-fluorescent protein (GFP) from Aequorea victoria, β-glucuronidase, β-galactosidase, and biotin can be used as binding partner tags. Any antigen or epitope for which a substantially pure antibody can be isolated or produced can be used as a tag according to the present invention" (emphasis added, page 56, line 4 – page 57, line 7).

In addition, the detector molecules can comprise reporter molecules other than fluorescent labels.

"The detector molecule further comprises a second region comprising a reporter region. A reporter is a molecule which can be conjugated or otherwise attached (i.e., covalently or non-covalently) to a the first region of the detector molecule. A reporter region can comprise more than one reporter molecule, such as at least 2, 3, 4, 5, or 10 reporter molecules. For example a reporter region can comprise

> two or more fluorochromes or two or more radioisotopes for signal enhancement. Reporters include fluorochromes such as phycoerythin (PE.), phycoerythrin-cyanin dye 5 (PECy5), rhodamine, and fluorescein isothiocyanate (FITC). Fluorescent protein tags, such as green fluorescent proteins, blue fluorescent proteins, red fluorescent proteins and variants thereof can be used. **Other suitable detectable reporters include those useful in colorimetric enzyme systems, e.g., horseradish peroxidase (HRP), alkaline phosphatase (AP), hexokinase in conjunction with glucose-6-phosphate dehydrogenase, β-galactosidase, and glucose oxidase. Radioactive compounds or elements, such as radioactive amino acids, radioisotopes, or radionuclide containing molecules can also be used as reporters.** The reporter molecules used may be readily selected by one of skill in the art and are not a limitation on the present invention. (page 62, line 6 – 19).

Therefore, Applicants submit that many configurations of tags exist which do not necessarily involve the use of three fluorescent labels, as claim 12 is currently written. Furthermore, energy transfer to multiple fluorescent labels was known at the time the application was filed (see, for example, Chapter 15. *Energy Transfer to Multiple Acceptors in One, Two or Three-Dimensions*. Principles of Fluorescence Spectroscopy Second Edition, Lakowicz, ed. Kluwer Academic/Plenum Publishers, June 1999). As such, Applicants submit that claim 12 is definite as written, and respectfully request withdrawal of the §112 rejection and reconsideration of the claim.

Rejections under 35 U.S.C. §102

1-4, 7, 9, 10, 11, 13, 28 and 32-34 are rejected under 35 U.S.C. §102(e) as being anticipated by Heroux et al. (U.S. Pat. No. 6,312,896). The Office Action states:

> "Heroux et al. disclose methods for measuring the activity of enzyme. Heroux et al. disclose combining an immobilized substrate and a substrate molecule with an enzyme. Heroux et al. disclose that the enzyme causes a change in the substrate molecule (for example phosphorylation of a protein) that induces the substrate to bind to a second molecule (col. 11, lines 1-62). Heroux et al. disclose that the substrate can be polypeptides and that these polypeptides can contain natural and unnatural units (col. 12, lines 1-32). Heroux et al. disclose that the substrates can be labeled (tagged) with ECL labels. Heroux et al. disclose an embodiment in which an immobilized substrate (binding partner) is combined with two tagged substrates and an enzyme (one tagged substrate and one detector molecule). Heroux et al. disclose that the second tagged substrate (detector molecule) binds with the first tagged substrate (tagged binding partner) (See Figure 6d). Heroux et

al. disclose that the invention can be used to assay an enzyme inhibitor (modulator) and/or to measure the inhibitory activity of test compound (col. 14). Heroux et al. disclose that FRET assays can be used to study the binding events (col. 19). Heroux et al. disclose that the detectable label can be a radioisotope (radioactive molecule)."

Applicants submit that claims 2-4, 7, 9, 10, 11, 13, 28 and 32-34 depend from claim 1. With this Amendment, Applicants have amended claim 1 to recite:

1. A method for monitoring activity of one or more enzymes comprising the steps of:
   A. mixing:
      (i) one or more tagged binding partner polypeptides;
      (ii) one or more binding partner polypeptides that bind to said one or more tagged binding partner polypeptides of (i); and
      (iii) one or more enzymes that add or remove a moiety to or from said one or more binding partner polypeptides or one or more tagged binding partner polypeptides;
      wherein said one or more tagged binding partner polypeptides or said one or more binding partner polypeptides comprise one or more sites for the addition or removal of said moiety, wherein addition or removal of said moiety promotes binding of said one or more binding partner polypeptides with the corresponding one or more tagged binding partner polypeptides; under conditions which promote binding of said one or more binding partner polypeptides with said one or more tagged binding partners; and
   B. detecting said binding, wherein the step of detecting binding comprises adding one or more detector molecules comprising a first region that associates with a tag of a said tagged binding partner polypeptides and a second region comprising one or more reporter molecules, wherein detection of binding as a result of said mixing is indicative of enzyme activity.

Applicants submit that Heroux does not anticipate claim 1 as currently amended. Heroux does not disclose a detector molecule which associates with the tag of the tagged binding partner polypeptide, as is required in claim 1. As previously mentioned and as discussed by the Examiner, Heroux discloses that the second tagged substrate (detector molecule) binds with the first tagged substrate. However, Heroux does not disclose a detector molecule that comprises "a first region that associates with a said **tag of said** one or more tagged binding partner polypeptides", as is required in claim 1.

Because Heroux fails to disclose a detector molecule that is not a substrate, for the enzyme whose activity is monitored, and because Heroux does not disclose a detector molecule that associates with the tag of the tagged binding partner, Applicants submit that Heroux does not anticipate claim 1 or claims 2-4, 7, 9, 10, 11, 13, 28 and 32-34 which depend from claim 1. As such, Applicants respectfully request withdrawal of the §102(e) rejection and reconsideration of these claims.

Rejections under 35 U.S.C. §103

Claim 5 is rejected under 35 U.S.C. §103(a) as being unpatentable over Heroux et al. in view of Levin et al. (U.S. 2002/0197696). The Office Action states:

> "Heroux et al. differ from the instant invention in failing to teach monitoring the rate of diffusion of the fluorescent molecule. Levin et al. disclose Fluorescence Correlation Spectroscopy (FCS), which measure the average diffusion rate of a fluorescent molecule within a small sample volume. Levin et al. disclose that FCS can be applied to protein-ligand interaction (p. 13, paragraph 0143).
>
> It would have been obvious to one of ordinary skill in the art to incorporate Fluorescence Correlation Spectroscopy as taught by Levin et al. into the modified method of Blau et al. because Levin et al. teaches that FCS provides for the average diffusion rate of a fluorescent molecule within a small sample volume and that it can be applied to protein-ligand interactions."

Applicants submit that claim 5 depends from claim 1. Applicants submit that, as discussed previously, Heroux et al. does not teach or suggest a detector molecule "associates with a tag of said tagged binding partner polypeptides." Therefore, Heroux et al. does not anticipate claim 1 of the present invention. This deficiency is not remedied by Levin. As such, Applicants submit that the combination of Heroux et al. and Levin are insufficient to render dependent claim 5 of the present invention obvious. Applicants therefore respectfully request withdrawal of the §103(a) rejection and reconsideration of claim 5.

Claims 8, 27 and 30 are rejected under 35 U.S.C. 103(a) as being unpatentable over Heroux et al. in view Colyer et al. (WO 99/11774). The Office Action states:

> "Heroux et al. differ from the instant invention in failing to specifically teach the one or more sites comprise a sequence, which directs modification by an enzyme such as kinase or phosphatase."
>
> "Colyer et al. disclose a method for monitoring the activity of an enzyme comprising monitoring the addition or removal of a moiety (p. 15). Colyer et al. disclose that the site contains an amino acid sequence, which is recognized by a post-translational modifying enzyme. Colyer et al. also disclose that the modifying enzyme can be a kinase or phosphatase (p. 17). Colyer et al. teaches that the use of such enzymes provides for efficient."
>
> "It would have been obvious to one of ordinary skill in the art to the use of enzymes as taught by Colyer et al into the method of Heroux et al because Heroux et al specifically teaches the enzyme causes a change in the substrate molecule (for example phosphorylation of a protein) (col 11, lines 1-62) and Colyer et al teaches that the use of such enzymes provides for efficient means of monitoring of post-translational modification of a protein."
>
> "With respect to the detector molecule being pre-bound to the one or more tagged binding partner polypeptides as recited in the instant claims. It would have been obvious to one or ordinary skill in the art to premix the detector molecule and the tagged binding partner before mixing with the binding partner polypeptide because premixing would allow for a longer incubation and would also allow for incubation without the presence of interfering substances"

As discussed above, Applicants submit that the combination of Heroux et al. and Colyer et al. does not supply all the elements needed to render the present invention obvious. As discussed above, Heroux et al. fails to teach or suggest a detector molecule that "associates with a tag of said tagged binding partner polypeptides." This deficiency is not remedied by Colyer et al. Therefore, Applicants submit that the combination of Heroux et al. and Colyer et al. does not render the present invention obvious.

Applicants further submit that claim 8 recites "the method of claim 1 wherein one or more detector molecules are pre-bound to the one or more tagged binding partner polypeptides." It is unclear to the Applicants what bearing this has on sites for post-translational modification by an enzyme as stated in the Office Action, and requests clarification on this point.

Concerning the assertion that "[i]t would have been obvious to one or ordinary skill in the art to premix the detector molecule and the tagged binding partner before mixing with the binding partner polypeptide because premixing would allow for a longer incubation and would

also allow for incubation without the presence of interfering substances", Applicants are not clear which prior art the Examiner is relying on for his argument, and respectfully request clarification. Regardless, given the lack of all elements in the combined references as discussed above, Applicants submit that the claimed invention is not obvious over the cited references. As such, Applicants respectfully request withdrawal of the §103(a) rejection and reconsideration of these claims.

Claims 51 and 52 are rejected under 35 U.S.C. §103(a) as being unpatentable over Heroux et al. in view of Colyer. The Office Action states:

> "Heroux et al differ from the instant invention in failing to specifically teach detecting binding of the binding partner polypeptides and the tagged binding partner polypeptides in both the presence and absence of candidate modulators."
>
> "Colyer et al disclose a method for monitoring the activity of an enzyme comprising monitoring the addition or removal of a moiety (p 15). Colyer et al disclose methods for screening a candidate modulator (p. 17-18). Colyer et al also teach detecting binding of the binding partner in both the presence and absence of a candidate modulator (p. 18). Coyler et al discloses this provides for an efficient means of monitoring and/or modulating post-translational modification (p 5)."
>
> "It would have been obvious to one or ordinary skill in the art to perform the method of Heroux et al in both the presence and absence of a candidate modulate as taught by Coyler et al because Heroux et al specifically teaches their method can be used to assay an enzyme inhibitor (modulator) and/or to measure the inhibitory ability of test compound and further because Coyler shows that detecting the binding or the binding partner in both the presence and absence of a candidate modulator provides for an efficient means of monitoring and/or modulating post-translational modification."

As discussed above, Applicants submit that the combination of Heroux et al. and Colyer et al. does not supply all the elements needed to render the present invention obvious, since neither teaches or suggests a detector molecule that "that associates with said tag of said one or more tagged binding partner polypeptides."

With this Amendment, Applicants have made an earnest effort to respond to all issues raised in the Office Action of April 26, 2004, and to place all claims presented in condition for allowance. Applicants submit that in view of the preceding remarks, all issues relevant to

patentability raised in the Office Action have been addressed. Applicants respectfully request the withdrawal of rejections over the claims of the present invention. If the Examiner believes that a telephone conversation with Applicant's attorney/agent would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney/agent of record.

Respectfully submitted,

Date: October 26, 2004

Name: Kathleen Williams
Registration No.: 34,380
Customer No.: 29933
Palmer & Dodge LLP
111 Huntington Avenue
Boston, MA 02199-7613
Tel: 617-239-0100